

# Complexation in Polyelectrolyte Solution with Divalent Surfactant

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## Abstract

We study a simple model of DNA divalent cationic surfactant complexation. We find that the combination of electrostatic and hydrophobic effects leads to a cooperative phenomenon in which as the amphiphile is added to the solution containing DNA, a large fraction of the DNA's charge is neutralized by the condensed divalent cationic surfactants, forming the surfoplex. This binding transition occurs for concentrations that are lower for divalent than for monovalent surfactants. Since the electrostatic strength is larger in the first case and the amount of surfactant lower, we suggest that multivalent amphiphilic molecules would be more efficient than monovalent for transfection.

*Key words:*

*PACS:* core-softened potential, diffusion

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## 1 Introduction

Gene therapy represents a promising way for the treatment of both genetic and acquired diseases. The basic idea is to replace the sick gene with a healthy one or in some cases to add a new gene to get a resulting synthesis of a therapeutic protein. The process of getting the new gene to its target involves the

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crossing of several barriers, such as the cell membrane and the nuclear membrane. Since both the DNA and the cell membranes are negatively charged, the naked polynucleotides are electrostatically prevented from entering into the cell. Viral vectors such as retroviruses and adenoviruses are very efficient and able to target a wide range of cells [1][2]. The gene transfection is accomplished by the use of a virus in which the native DNA has been replaced by the required DNA. Since the main purpose of the virus is to replicate itself, the new gene is successfully transfected to the cell nucleus by endocytosis or membrane fusion. This process suffers from the drawback that in cases where repeated treatment is needed, this might cause the immune system to react negatively due to the viral origin of the vector.

As a solution for this problem nonviral vectors have been developed. One of the approaches, pioneered by Felgner and Ringold [3][4] relies on the association between anionic nucleic acid and cationic lipid liposomes. The process of association neutralizes the excess negative charge of the DNA and the DNA-liposome penetrate into the cell by endocytosis. The efficiency of this method is low and they are toxic to the cell at the concentrated used.

Among the parameters needed for achieving efficient transfection is the requirement that the complex should have small size similar to a virus [5]. This factor indeed limits the efficiency of the DNA-liposomes complexes. In contrast, cationic surfactant have been shown to condense to DNA into discrete particles containing a single nucleic acid molecule [6][7] that can neutralize and revert the charge of the DNA [8]-[11] allowing the surfoplex to approach the membrane. Monovalent DNA-surfactant complexes exhibit a discrete first-order phase transition between elongated coil and collapsed globule [12] for a concentration of amphiphilic molecules well below the miscelar concentration. Despite this unique feature, monovalent detergent are poorly efficient in vitro in gene transfer [13][14]. When the surfoplex approaches the membrane, the interaction between the surfactant molecules and the phospholipid bilayer overcomes the electrostatic attraction between the anionic nuclei acid and the cationic detergent. The surfactant incorporate into the phopholipid membrane resulting in the unfolding of the DNA that stays outside the cell [15].

Therefore, in order to have a stable surfoplex, one needs to enhance the electrostatic interaction. In this paper we present a model of DNA-amphiphilic solution where the surfactant is divalent. We find that in equilibrium, solution consists of complexes composed of DNA and associated counterions and amphiphiles. Even for an small amount of amphiphiles in the solution, the cooperative binding is found. Due to the high valence, the interaction between the nuclei acid and the detergent is expect to overcome the interaction between the surfactant molecules and the phospholipids in the membrane and stay associated to the DNA allowing it to transpass the membrane. The complex formed is more stable than the monovalent structure, forming a collapsed

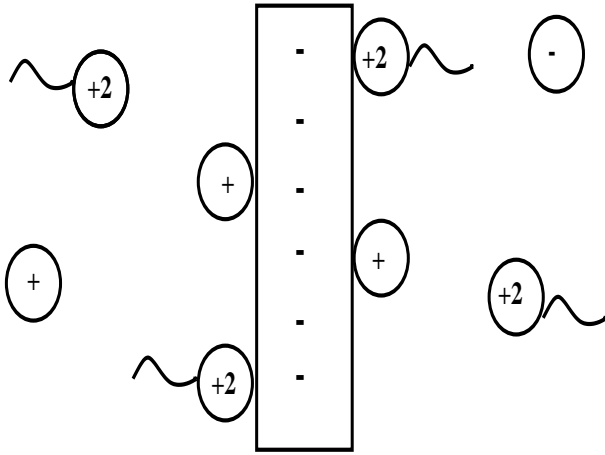


Fig. 1. The model

globule that we expect will transect with high efficiency [16].

## 2 The Model

Our model, illustrated in Fig. 1, consists of a solution of *DNA* strands of length  $L$  and diameter  $a_p$ , divalent surfactant and monovalent salt. In aqueous solution, the polyions become ionized resulting in a negative charge  $-Zq$  distributed uniformly distributed with separation  $b = L/Z$ . The solvent, water, is modeled as a continuous medium of dielectric constant  $D$ . The ions of the salt are completely dissociated, forming an equal number of positive and negative ions. Similarly, the surfactants are assumed to be fully dissociated producing negative monovalent coions and polymeric chains with a divalent cationic head group. For simplicity, all the counterions and coions are treated as identical, independent of the molecules from which they were derived. The electrolytes are depicted as hard spheres of diameter  $a_c$  and charge  $\pm q$  and the surfactant is modeled as a polymer of  $s$  monomers each one considered as a rigid sphere of diameter  $a_c$  with the head monomer carrying a charge of  $+2q$ .

The interaction between the hydrophobic tails is short ranged and characterized by the parameter  $\chi$ . The density of DNA strands is  $\rho_p = N_p/V$ , the density of monovalent salt is  $\rho_s = N_s/V$  and the density of divalent amphiphiles is  $\rho_a = N_a/V$ .

The strong electrostatic interactions between the polyions, the counterions, salt cations and surfactant heads leads to the formation of complexes, which in thermodynamic equilibrium will be made up of one polyion,  $n_c$  monovalent counterions and  $n_a$  divalent surfactant. We do not consider the effects of polydispersity in the size of the complexes, since it does not affect the final

result. Due to the association and to the charge conservation, there are only two free quantities and so,

$$\rho_c = (Z - n_c)\rho_p + \rho_s \quad \rho_{a+} = \rho_a - n_a\rho_p \quad \rho_- = \rho_s + 2\rho_a \quad (1)$$

where  $\rho_c$  is the density of free monovalent counterions,  $\rho_{a+}$  is the density of free amphiphiles and  $\rho_-$  is the density of negative ion.

The objective of this theory is to determine the number of counterions  $n_c$  and surfactants  $n_a$  associated to each *DNA* strand. For this, we construct the Helmholtz free energy of the system and minimize it. The details about the model can be found elsewhere [8]-[11]. We give here the main steps. The relevant contributions for the Helmholtz free energy are two, the electrostatic and the entropic namely:

$$F = F_{el} + F_{ent} \quad (2)$$

In  $F_{el}$  three types of interactions can be found: between the free ions and free surfactants,  $F_{is}$ , between the complex, free ions and free surfactants,  $F_{pis}$ , and between the complexes  $F_{pp}$ .

$$F_{el} = F_{is} + F_{pis} + F_{pp} \quad (3)$$

With the aid of the theory of Debye-Hückel-Bjerrum (DHBj) it is possible to find the electrostatic interaction between the complexes, ions and surfactant given by [8]-[11]:

$$\beta f^{pis} = -\frac{\rho_p Z_c^2 (a/L)}{T^* (\kappa a)^2} \left\{ 2 \ln [\kappa a K_1(\kappa a)] - I_0 + \frac{(\kappa a)^2}{2} \right\} \quad (4)$$

with  $\beta = 1/k_B T$  and

$$I_0 \equiv \int_0^{\kappa a} \frac{x K_0^2(x)}{K_1^2(x)} dx \quad (5)$$

where  $\kappa$  in  $(\kappa a)^2 = 4\pi\rho_1^*/T^*$  is the inverse of the Debye screening length,  $\rho_1^* = \rho_1 a^3 = \rho_c a^3 + 4\rho_{a+} a^3 + \rho_- a^3$  is the reduced density and  $T^* = Dk_B T a / q^2$  is the reduced temperature. Furthermore,  $Z_c = Z - n_c - 2n_a$  is the valence of each complex and  $a = (a_c + a_p)/2$  is the effective radius of the exclusion cylinder around each complex.

In the framework of the Debye-Hückel theory, the interaction between the free ions is given by:

$$\beta f^{is} = -\frac{1}{4\pi a_c^3} \left[ \ln(1 + \kappa a_c) - \kappa a_c + \frac{(\kappa a_c)^2}{2} \right]. \quad (6)$$

The electrostatic free energy interaction between two complexes for large separations is screened. The short-range of this interaction allows one to use a mean-field approximation resulting in:

$$\beta f^{pp} = \frac{2\pi a^3 Z_c^2 \rho_p^2 \exp(-2\kappa a)}{T^*(\kappa a)^4 K_1^2(\kappa a)}. \quad (7)$$

The calculation of the entropic contribution can be obtained with the aid of Flory [17] theory of mixing. The free energy is a sum of ideal free energies of various species, namely

$$\beta f^{ent} = \sum \left[ \rho_s - \rho_s \ln \left( \frac{\phi_s}{\zeta_s} \right) \right] \quad (8)$$

where  $s$  represents the different species and  $\zeta_s$  is the internal partition of the species  $s$ . In the case of the particles without structure, the internal partition function  $\zeta_- = \zeta_c = \zeta_{a+} = 1$ . The volume fraction  $\phi_s$  of the different species are:

$$\begin{aligned} \phi_p &= \frac{\pi \rho_p^*}{4(a/L)} \left( \frac{a_p}{a} \right)^2 + \frac{Z\pi \rho_p^*}{6} (s_a m_a + m_c) \left( \frac{a_c}{a} \right)^3 \\ \phi_c &= \rho_c^* \frac{\pi}{6} \left( \frac{a_c}{a} \right)^3 \\ \phi_{a+} &= \frac{s_a \pi \rho_{a+}^*}{6} \left( \frac{a_c}{a} \right)^3 \\ \phi_- &= \frac{\pi \rho_-^*}{6} \left( \frac{a_c}{a} \right)^3. \end{aligned} \quad (9)$$

Here we introduce the fractions counterions,  $m_c = n_c/Z$ , and surfactant,  $m_a = n_a/Z$ , associated to each DNA strand. Accounting for all these terms, the entropic contribution becomes

$$\begin{aligned} \beta f^{ent} &= \rho_p \ln \left( \frac{\phi_p(1 + m_c + m_a)}{\zeta_{cl}(1 + m_c + m_a s_a)} \right) - \rho_p \\ &\quad + \rho_c \ln \phi_c - \rho_c + \rho_{a+} \ln \frac{\phi_{a+}}{s_a} - \rho_{a+} \\ &\quad + \rho_- \ln \phi_- - \rho_- \end{aligned} \quad (10)$$

where the entropy for the free surfactants arises from the Flory theory for polymers [17] which is also the basis of the entropic contribution for the complex

[8]. The internal partition function of the complex,  $\zeta_{cl}$ , can be calculated by modeling the DNA by an one dimensional lattice with  $Z$  sites. If the number of associated ions to each site can be only zero or one, this problem becomes equivalent to finding the free energy of an one dimensional array with the three different states.

Due to the presence of two different valences, the exact solution of this model is not trivial, so we employ the mean-field Gibbs-Bogoliubov-Feynman inequality. The resulting partition function is given by

$$\begin{aligned}
 -\ln \zeta_c [m_c, m_a] = & \xi K \left[ \frac{Z_c^2}{Z^2} - 1 \right] + \beta \chi (Z - 1) m_a^2 \\
 & + Z m_c \ln m_c + Z m_a \ln m_a \\
 & + Z (1 - m_c - m_a) \ln (1 - m_c - m_a)
 \end{aligned} \tag{11}$$

where  $\xi \equiv \beta q^2 / Da$  is the Manning parameter,  $K = Z[\psi(Z) - \psi(1)] - Z + 1$ , and  $\psi(n)$  is the digamma function.

The equilibrium configuration of the system is found by the minimization of the Helmholtz free energy, leading to two equations, namely

$$\begin{aligned}
 \frac{\partial F}{\partial m_c} \delta m_c &= 0 \\
 \frac{\partial F}{\partial m_a} \delta m_a &= 0.
 \end{aligned} \tag{12}$$

Solving this system of two equations, it is possible to obtain the values of  $m_c$  and  $m_a$ .

### 3 Results and Conclusions

We define a "surfoplex" to be a complex in which almost all of the DNA's phosphate groups are neutralized by the associated surfactant molecules. As mentioned earlier, we are interested in the minimum amount of cationic surfactant needed to transform naked DNA into surfoplexes. To this effect, we study the dependence of the number of condensed surfactant molecules on the bulk concentration of surfactant  $\rho_a$ . In order to evaluate the relevance of hydrophobic interactions between the amphiphiles, the hydrophobic parameter was varied from 0 to  $\beta \chi = -6$ . The effect of addition of high concentrations of salt to the system was analyzed by varying the amount of salt added to the system  $\rho_s$ .

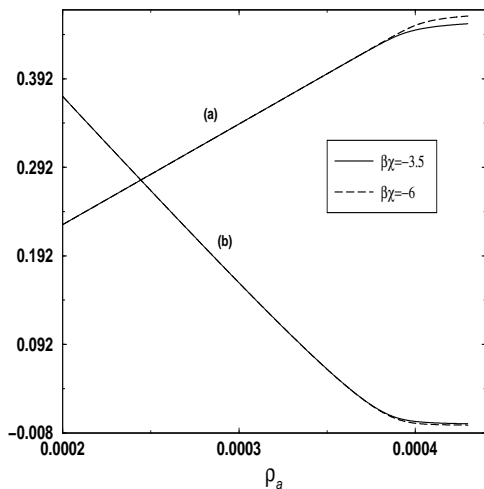


Fig. 2. Effective binding of amphiphiles (a)  $m_a$  and counterions (b)  $m_c$  as a function of the surfactant concentration  $\rho_a$ . The density of DNA and salt are respectively  $2 \times 10^{-6} M$  and  $10^{-4} M$ . The hydrophobicity are  $\beta\chi = -3.5$  (solid line) and  $\beta\chi = -6$  (dashed line).

Fig.2 and Fig.3 illustrate the surfactant and the counterion binding isotherm,  $m_a$  and  $m_c$  respectively, as a function of total amphiphilic concentration for two different hydrophobicity parameter  $\beta\chi = -3.5, -6$  and salt concentrations  $\rho_s = 10^{-3} M, 10^{-4} M$ . At very low densities of divalent surfactant, the condensation is dominated by the monovalent ions, since the divalent amphiphilic molecules gain more entropy, and thus lower the total free energy, by staying free. However, as the concentration of divalent surfactant increases, the gain in electrostatic energy due to condensation and to the hydrophobicity of the amphiphilic molecules wins over the entropy.

We note that unlike the association with the ionic monovalent surfactants, which exhibits a large degree of cooperativity characterized by the sharp rise in the surfactant binding fraction, the replacement of the condensed monovalent counterions by the divalent surfactant proceeds more smoothly. This result could have been anticipated a priori. After the first amphiphile is associated, the condensation of additional molecules is energetically favored since the buildup of the hydrocarbon density in the vicinity of a polyion helps to exclude water and, thus, reduces the unfavorable hydrophobic energy of the alkyl tails. This effect competes with the electrostatic repulsion between the like-charged counterions that tends to inhibit it any further association. The repulsion is stronger for divalent ions than for monovalents and this ex-

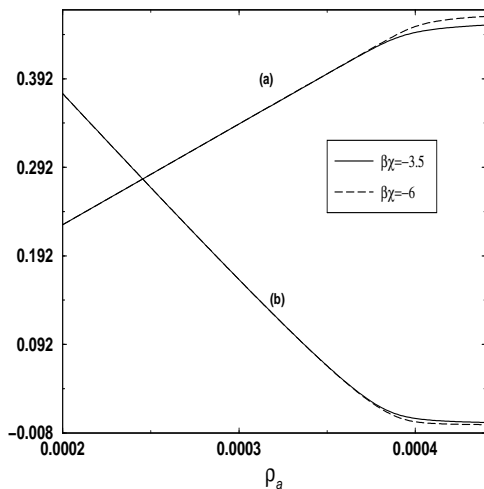


Fig. 3. The same as Fig.2 for  $\rho_s = 10^{-3}M$ .

plain why the cooperative effect is stronger in the later. In the case of the divalent amphiphilic molecules the surfoplex is formed for a density of surfactant around  $0.0005M$  as illustrated in Fig. 4 what is much lower than the one observed in the monovalent case [8]-[9]. For comparison with the case of monovalent surfactant illustrated in refs. [8] for producing Fig. 4 we employ the hydrophobicity factor  $\beta\chi = -4$ .

Fig. 5 and Fig 6 illustrates the effective charge of the complex. Due to the simplicity of our model that does not allow for the association of more than one surfactant molecule to each charged group along the DNA, there is no charge inversion. However, the decrease in the charge produced by the divalent surfactant is larger than the one observed when the amphiphilic molecules are monovalent for a similar model [8]-[9].

In resume, we have presented a simple theory of DNA, for monovalent salt and divalent surfactant solutions. Our results should be of direct interest to researchers working on the design of improved gene delivery systems. In particular, we find that addition of cationic divalent surfactants leads to a cooperative binding. This binding happens far below the critical micell concentration and far below the concentration for this transition to happen in the presence of monovalent surfactant. Until now experimental attempts in employing surfactants as nonviral agent for transfection have been limited to the use of monovalent surfactants. The surfoplex produced with monovalent amphiphilic exhibit poor efficiency. Close to the membrane, most of the surfactants disassociate from the DNA and the transfection does not occurs [13][14]. We propose the use of divalent cationic surfactants in the formation of the complex. Besides being more strongly connected to the charged groups of the



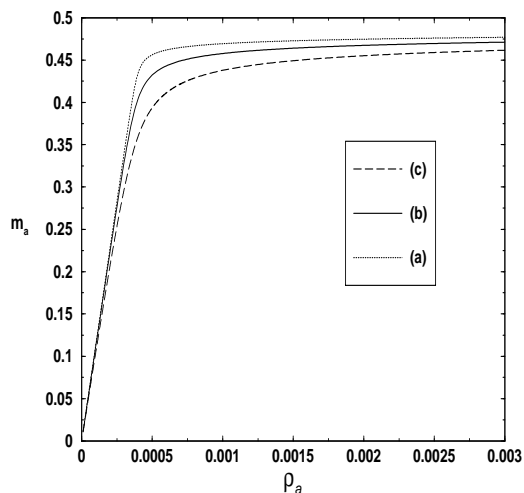


Fig. 4. Effective binding of divalent surfactant  $m_a = n_a/Z$  and counterions  $m_c = n_c/Z$  as a function of the surfactant concentration  $\rho_a$  for various salt concentrations: (a)  $5mM$ , (b)  $18mM$  and (c)  $40mM$ . The density of DNA is  $2 \times 10^{-6}M$  and the hydrophobicity is  $\beta\chi = -4$ .

DNA what will give more stability to the complex during the transfection, the amount of surfactant required is lower. Since the surfactant are toxic to the organism, this should reduce the risk of unnecessary medical complications.

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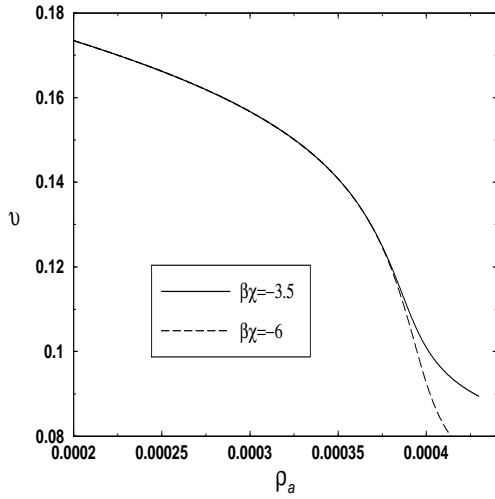


Fig. 5. The effective charge per charged group of the DNA,  $v = 1 - 2m_a - m_c$  a function of the surfactant concentration  $\rho_a$ . The density of DNA and salt are respectively  $2 \times 10^{-6} M$  and  $10^{-4} M$ . The hydrophobicity are  $\beta\chi = -3.5$  (solid line) and  $\beta\chi = -6$  (dashed line)

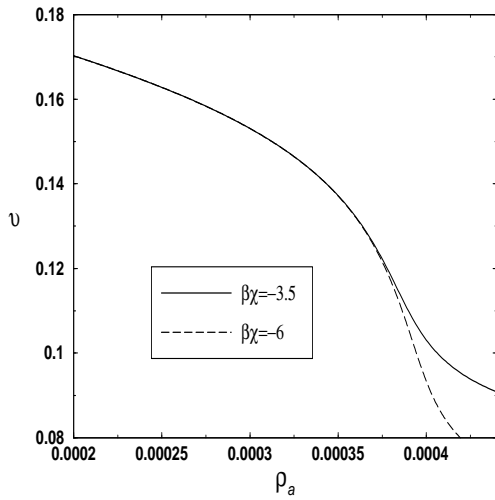


Fig. 6. The same as Fig. 5 for  $\rho_s = 10^{-3} M$

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